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Intraorganellar enzyme partitioning: a novel layer of chlorophyll biosynthesis regulation

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Chlorophyll is the most abundant natural pigment on Earth and is the key component of photosynthesis, ultimately providing oxygen and energy for us all. The ability of chlorophyll to absorb sunlight and to convert solar to chemical energy is vital, but at the same time, due to its unique photodynamic properties, chlorophyll can be harmful. Therefore, the metabolism of chlorophyll in plants is tightly regulated. The first committed step of chlorophyll biosynthesis in plants is the formation of δ-amino levulinic acid (ALA) from glutamate, for the subsequent synthesis of a variety of tetrapyrrole pigments, including chlorophyll, heme and phytochromobilin. The key enzyme of ALA synthesis, glutamyl-tRNA reductase (GluTR), has been shown in the past to be regulated in a complex manner. This involves transcriptional control of tissue- and developmental-specific expression of different GluTR-encoding *HEMA* genes as well as posttranslational control by different proteins, such as FLUORESCENCE (FLU) (Meskauskiene et al. 2001), a negative feedback regulator of protochlorophyllide formation, GluTR-binding protein (GBP) (Czarnecki et al. 2011), which likely balances relative levels of chlorophyll and heme, and the chloroplast signal recognition particle 43 (SRP43) (Wang et al. 2018), which coordinates light harvesting complex and chlorophyll assembly and thylakoid membrane integration. In addition, GluTR was shown to be a target for degradation by the Clp protease system (Apitz et al. 2016).

In this issue, Schmied et al. (2018) add an additional layer of regulation of GluTR, i.e. the intraplastidic partitioning between stroma and thylakoid membrane. The authors used a rapid method of separation of chloroplast stroma and membrane fractions followed by immunoblot analysis to quantify relative abundances of GluTR under different growth conditions, light/dark regimes and in different genetic backgrounds. In plants grown under varying light intensities for two weeks, i.e. low light, normal light and high light (10, 120 and 300 µmol photons $m^{-2}s^{-1}$, respectively), stroma-localized GluTR faction (sGluTR) and ALA synthesizing capacity (ASC) correlated well. Likewise, during development under either short day conditions or in continuous light, ASC and sGluTR correlated and peaked after two weeks. GluTR levels and activity are known to be low in dark-grown plants but to massively increase upon illumination and, inversely, to diminish when light-grown plants are transferred to extended darkness (Kobayashi and Masuda 2016). The authors analyzed the distribution of GluTR between stroma and membrane fraction under such conditions. Under de-etiolation, sGluTR increased rapidly and greatly, while the membrane-localized GluTR faction (mGluTR) changed much less dramatically. Analogously, when exposing continuous light-grown plants to an extended phase of darkness, sGluTR decreased rapidly within a few hours, while decline of mGluTR was much less pronounced. Again, this indicated that the rapid occurrence of chlorophyll upon de-etiolation and the known decrease of ASC during darkness (Richter et al. 2010) correlate with the stromal fraction of GluTR, but not with the membrane fraction. Finally, the authors analyzed mutants that are defective in proteins known to interact with GluTR, i.e. *flu1* (Meskauskiene et al. 2001), *gbp* (Czarnecki et al. 2011) and *clpC1*, a mutant deficient in a subunit of the Clp protease

that has been shown to directly interact with GluTR (Apitz et al. 2016). In addition, they analyzed a *hema1* mutant, complemented with a truncated version of GluTR (ΔHBD), lacking the N-terminal part of the protein, the so-called heme-binding domain (HBD). This domain is the site of interaction with GBP and ClpC (Apitz et al. 2016) and, thus, over-accumulates GluTR because of increased protein stability and lack of proteolytic degradation upon extended darkness. The intrachloroplast distribution of GluTR was unaltered in the absence of GBP, but sGluTR was increased in *clpC1* and in the ΔHBD line.

Based on the data presented in Schmied et al. (2018), a model can be drawn (Fig. 1) that indicates activity of GluTR, among other mechanisms, to be regulated by (reversible) binding to the thylakoid membrane. This idea has been put forward in the past, i.e. with the finding that FLU can directly interact with GluTR (Kauss et al. 2012). Indeed, as shown in Schmied et al. (2018), absence of FLU in the *flu* mutant, caused detachment from the membrane of almost the entire GluTR fraction, combined with slower degradation during extended darkness, which may be the reason for more GluTR activity combined with increased accumulation of protochlorophyllide in *flu* in the dark. The application of a rapid method of separation of soluble and membrane-bound protein fractions combined with sensitive immunoblot analysis, allowed Schmied et al. (2018) to clearly correlate ASC to the stromal fraction of GluTR. Nevertheless, the findings of Schmied et al. (2018) leave many open questions that await further investigation. For instance, how is the physical, protochlorophyllide-dependent contact between FLU and GluTR established such that it attaches the latter to the protein and how does this abolish GluTR activity? What is the precise role of GBP in this respect? Schmied et al. (2018) show that the relative distribution between stroma and membrane are rather similar for GBP and GluTR. GBP has been ascribed a protective role for GluTR and, thus, to antagonize the proteolytic activity of Clp (Apitz et al. 2016). Would GBP have this role also at the thylakoid membrane, despite the fact that in *gbp*, mGluTR is also increased compared to wild type (Schmied et al. (2018)?

The regulation of tetrapyrrole biosynthesis in plants is intriguing and highly complex. The work of Schmied et al. (2018) contributes an important aspect of the control of ALA biosynthesis, i.e. spatial separation of GluTR as a regulator of its activity. Future analyses are required to elucidate this aspect further, especially given the fact that many of the intermediates of chlorophyll biosynthesis are potentially phototoxic, as observed by the cell death phenotypes occurring in mutants of different biosynthetic steps (Tanaka et al. 2011). Thus, metabolic channeling, enabled through physical contact between successive biosynthetic enzymes, possibly in the form of a multiprotein complex, is likely to occur. Where would such a multiprotein complex be located, and would intraplastidic protein relocation also regulate other enzymes of the pathway? While limited information is available for chlorophyll biosynthesis, metabolic channeling has been proposed during chlorophyll breakdown (Sakuraba et al. 2012), where occurrence of potentially phototoxic intermediates in respective mutants likewise exhibit premature cell death phenotypes (Tanaka et al. 2011).

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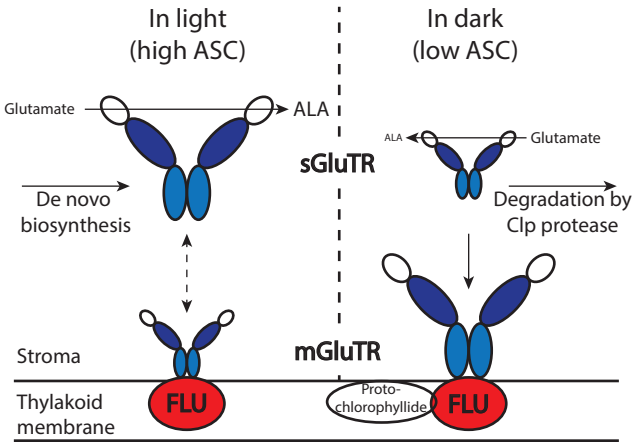
Figure legend:

Fig. 1. Model of intraplastidic partitioning of GluTR. In the light, *de novo* synthesis and localization of the major fraction of GluTR in the stroma (sGluTR) allows high δ-amino levulinic acid (ALA) synthesizing capacity (ASC) and chlorophyll formation. In the dark, ASC and sGluTR are low, because of re-location of GluTR to the thylakoid membrane (mGluTR) through protochlorophyllide-induced efficient binding to FLUORESCENCE (FLU) and through degradation by the Clp protease. GluTR, drawn as V-shaped dimer with the N-terminal, heme-binding domain (dark blue), the catalytic domain (white) and the C-terminal, FLU-binding domain (light blue).


In light
(high ASC)
In dark
(low ASC)
Glutamate
ALA
sGluTR
ALA
Glutamate
De novo
biosynthesis
Degradation by
Clp protease
Stroma
mGluTR
Thylakoid
membrane
FLU
Proto-
chlorophyllide
FLU